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Isomer-Specific Activity of Dichlorodyphenyltrichloroethane with Estrogen Receptor in Adult and Suckling Estrogen Reporter Mice

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We investigated the tissue-specific effects of dichlorodyphenyltrichloroethane (DDT) isomers in adult and suckling newborn mice, using a novel mouse line engineered to express a reporter of estrogen receptor transcriptional activity (ERE-tkLUC mouse). The DDT isomers p,p'-DDT [1,1,1-trichloro2,2-bis(p-chlorophenyl) ethane] and o,p'-DDT [1,1,1-trichloro-2(p-chlorophenyl)-2-(o-chlorophenyl) ethane] were specifically selected as a weak and a strong estrogen, respectively. In adult male mice, p,p'-DDT induced luciferase activity in liver, brain, thymus, and prostate but not in heart and lung. The effect of p,p'-DDT was dose-dependent, maximal at 16 h after sc treatment, and completely blocked by the estrogen receptor antagonist ICI-182,780. In all the organs analyzed, except the liver, administration of o,p'-DDT showed a pattern of luciferase induction superimposable to that of its isomer p,p'-DDT. In liver, o,p'-DDT significantly decreased basal luciferase activity and blocked the reporter induction by 17β-estradiol. These data lead us to hypothesize that a modulation of ER activity may be involved in the toxic effects of DDT demonstrated by epidemiological and experimental studies.

Luciferase activity was also studied in 4-d-old mice lactating from a mother injected with either p,p'-DDT or o,p'-DDT. Both isomers induced a 2-fold increase in the newborn brain. An opposite effect was observed in liver, where p,p'-DDT increased and o,p'-DDT decreased luciferase, thus indicating that these compounds modulate ER activity in adult and newborn tissues by use of a similar mechanism.

The ERE-tkLUC mouse proves to be a suitable tool to functionally assess the tissue specificity of estrogenic/antiestrogenic compounds in adult (as well as in suckling) mice. (*Endocrinology* 143: 4544–4551, 2002)

ORGANOCHLORINE PESTICIDES COMPOSE a large family of chemicals that are toxic for endocrine functions (1–6). The use of these compounds has been banned or severely restricted in most industrialized countries (7). However, because of their persistence in the environment and in living organisms, they still represent a largely unsolved problem for human health (8–12). Studies carried out over the last decade documented that these xenohormones bind to and activate the estrogen receptors (ERs) and, consequently, induce estrogenic effects in different animals. It is now clear that these compounds, also named endocrine disrupters (EDs), are able to evocate an endocrine response at the level of tissues target of sex steroids, thyroid, and adrenal hormones (13–15). This has been shown *in vitro* using cell lines engineered to overexpress specific receptors and reporter genes (16, 17) and *in vivo* with specific animal models in which some of the aspects of the pathophysiological action of these compounds have been illustrated (18–20). The recent recognition of the multifaceted activities of estrogen and its receptors reveals new potential targets for the activity of EDs. This, together with the novel acquired knowledge that, in the context of different tissues, a given ligand may have even opposite activity (*e.g.* agonist or antagonist) on ERs, imposes evaluation of the potential hazard of ER-interacting chemicals by the analysis of their effects on the transcriptional activity of ER in each specific target organ. The animal models applied to date to the study of the *in vivo* effects of organochlorine pesticides (21–23) do not allow us to investigate the toxicodynamic and toxicokinetic properties of these compounds in the whole organism and, in particular, in the nonreproductive organs. The availability of a novel line of mice reporter of ER transcriptional activation (ERE-tkLUC; Ref. 24) prompted us to prove its applicability to the study of EDs by evaluating the activity of the two isomers composing dichlorodyphenyltrichloroethane (DDT), a well-known weak environmental estrogen. We here provide a map of ER activation by the two DDT isomers, p,p'-DDT and o,p'-DDT in nonreproductive tissues and prostate and demonstrate that these two compounds may either block or activate this receptor transcriptional activity, depending on the tissue targeted. In addition, because of the high concern regarding the effective exposure of the newborn to chemicals through the mother's milk (25), we used our system to assess the extent of ER activation in tissues of suckling newborns breast-fed by DDT-exposed mothers.

Abbreviations: CNS, Central nervous system; DDT, dichlorodyphenyltrichloroethane; 17β-E2, 17β-estradiol; ED, endocrine disrupter; ER, estrogen receptor; ERE, estrogen-responsive element; GC, gas chromatography; LUC, luciferase; MS, mass spectrometry analysis; PR, progesterone receptor; tk, thymidine kinase.

Materials and Methods

Experimental animals

The procedures involving the animals and their care were conducted in accord with institutional guidelines, which comply with national and

international laws and policies (National Institutes of Health, Guide for the Care and Use of Laboratory Animals, 1996, 7th ed. Washington, D.C.; National Academy Press, National Research Council Guide, www.nap.edu/readingroom/books/labrats).

ERE-tkLUC transgenic mice were kept in animal rooms maintained at a temperature of 23 C, with natural light/dark cycles. For the experiments, we used heterozygous littermates obtained by mating our founders with C57BL/6 wild-type mice. Heterozygous transgenic male mice were screened by PCR analysis for the presence of the transgenic cluster.

Heterozygous male mice (2 months old) were injected ip with 100 μl 17β-E2, p,p′-DDT or o,p′-DDT at the needed concentration or with 100 μl of vehicle (vegetal oil) as control. At the time of death of the animals, by cervical dislocation, the tissues were dissected and immediately frozen on dry ice. Tissue extracts were prepared by homogenization in 500 μl of 100-mM K_2PO_4 lysis buffer (pH 7.8) containing 1 mM dithiothreitol, 4 mM EGTA, 4 mM EDTA, and 0.7 mM phenylmethylsulfonylfluoride, with three cycles of freezing-thawing and 30 min of microfuge centrifugation at maximum speed. Supernatants, containing luciferase, were collected, and protein concentration was determined by Bradford's assay (26).

Chemicals

We purchased 17β-estradiol (17β-E2) from Sigma (Pomezia, Italy). Radioligands for progesterone receptors (PRs) were purchased from Amersham (Milan, Italy). Organochlorine compounds p,p′-DDT [1,1,1,-trichloro-2,2-bis(p-chlorophenyl)ethane] and o,p′-DDT [1,1,1,-trichloro-2(p-chlorophenyl)-2-(o-chlorophenyl)ethane] were purchased from Superchrom (Milan, Italy). ICI 182,780 was a gift from Zeneca Pharmaceuticals (Cheshire, UK).

Enzymatic assay

Luciferase enzymatic activity was measured, as reported by de Vet *et al.* (27), in tissue extracts at a protein concentration of 1 mg/ml. The light intensity was measured with a luminometer (Murex Diagnostici, Rome, Italy) over 10 sec and expressed as relative light units per microgram proteins.

Ligand-binding assays

The PR concentration in the cytosols from liver was measured by the charcoal-dextran method, at a protein concentration between 1 and 2 mg/ml, and carried out by means of the multiple-saturation analysis (range of ^{3}H-ORG2058: 0.75–12 nM) in the presence or absence of a 100-fold excess of cold progesterone as competitor. The values were plotted by the method of Scatchard (28). Proteins in cytosol were determined by the method of Bradford (26).

Gas chromatography-mass spectrometry (GC-MS) analysis

Sample extraction. One hundred microliters of serum or 40 mg of milk were extracted with n-hexane/diethylether, 1:1 (vol/vol), mixture and eluted on a Florisil cartridge (6 ml, 1 g; Supelco, Bellefonte, PA) and a silica cartridge (6 ml, 1 g; Supelco) sequentially. The extracts were brought to dryness, and the derivatives were dissolved in n-hexane (100 μl).

GC-MS analysis. Two microliters of the extracts were injected in GC-MS. Then, p,p′-DDT was separated by chromatography on a PONA fused silica capillary column (Hewlett-Packard, Palo Alto, CA) with helium as carrier gas (flow-rate, 1 ml/min constant flow) and by temperature programming. The instrument used was a GC HP 6890 MS HP 5972-A (Hewlett-Packard); p,p′-DDT was identified by 235 and 237 ions and quantified by the standard addition method (10- and 100-ng/ml solutions). The concentrations are expressed as ng/ml or ng/g·milk fat.

Statistical analysis

P values were calculated with ANOVA followed by the Scheffé test.

Results

Tissue-specific modulation of transgene expression by p,p′-DDT

To determine the activity of the DDT isomer p,p′-DDT on ER, in the context of specific tissues, male mice were treated with p,p′-DDT using a route of administration (ip) and a dosage known, by preceding studies carried out *in vivo*, to be active on ER. In parallel, mice were also treated with 17β-E2 as a reference compound. Figure 1 shows that p,p′-DDT and 17β-E2 induced luciferase activity in liver, brain, prostate, and thymus but not in heart. The p,p′-DDT was also unable to induce luciferase in lung, an organ clearly responsive to the treatment with estradiol. With p,p′-DDT, the strongest luciferase induction was observed in liver (10-fold induction). In the other responsive organs, luciferase content was generally doubled, with respect to time 0 and to controls (brain and thymus, 2-fold induction; prostate, 1.6-fold induction). Interestingly, with p,p′-DDT, the peak of luciferase accumulation was observed 16 h after treatment. With 17β-E2, this effect was obtained 6 h after injection. Therefore, the ER response to p,p′-DDT seemed to be delayed, with respect to 17β-E2. Measurement of luciferase content at 6, 16, and 24 h of animals treated with vehicle demonstrated that the basal activity of the promoter is very stable in time.

At the dosage selected for the study, the extent of luciferase accumulation was always lower with p,p′-DDT than with estradiol administration. To investigate whether this was attributable to a different intrinsic activity of the two compounds or to the dosage used, we proceeded to compare the effects of estradiol and p,p′-DDT using a large range of concentrations. In all the tissues investigated, p,p′-DDT was maximally active at a dosage 100 times higher (5000 μg/kg) than that of 17β-E2 (50 μg/kg) (Fig. 2, A and B). Thus, in agreement with the previous observations, in all the tissues assayed, perhaps with the exception of brain, the intrinsic activity of p,p′-DDT never approached that of 17β-E2.

Luciferase activity reflects the effects of 17β-E2 and p,p′-DDT on an endogenous target gene, PR

To verify whether the modulation of the transgenic marker by 17β-E2 and p,p′-DDT mirrored that of an endogenous estrogen target gene, we measured PR binding in liver after the dose- and time-dependency treatments (Fig. 3). PR content, as measured by radioligand assay, was maximal at 6 and 16 h after 17β-E2 or p,p′-DDT injection, respectively. The doses determining the maximal response were 50 and 5000 μg/kg for the two compounds (Fig. 3A). In agreement with the observations on the effects of the two compounds on luciferase, 17β-E2 intrinsic activity was significantly higher, with an induction of PR of 20-fold *vs.* the 12-fold observed with p,p′-DDT. The basal levels of PR were undetectable at different time points (not shown). The measurement by immunoenzymatic assay of PR content in liver of mice treated for 0, 6, and 16 h with p,p′-DDT or 17β-E2 confirmed the results obtained by binding (not shown).

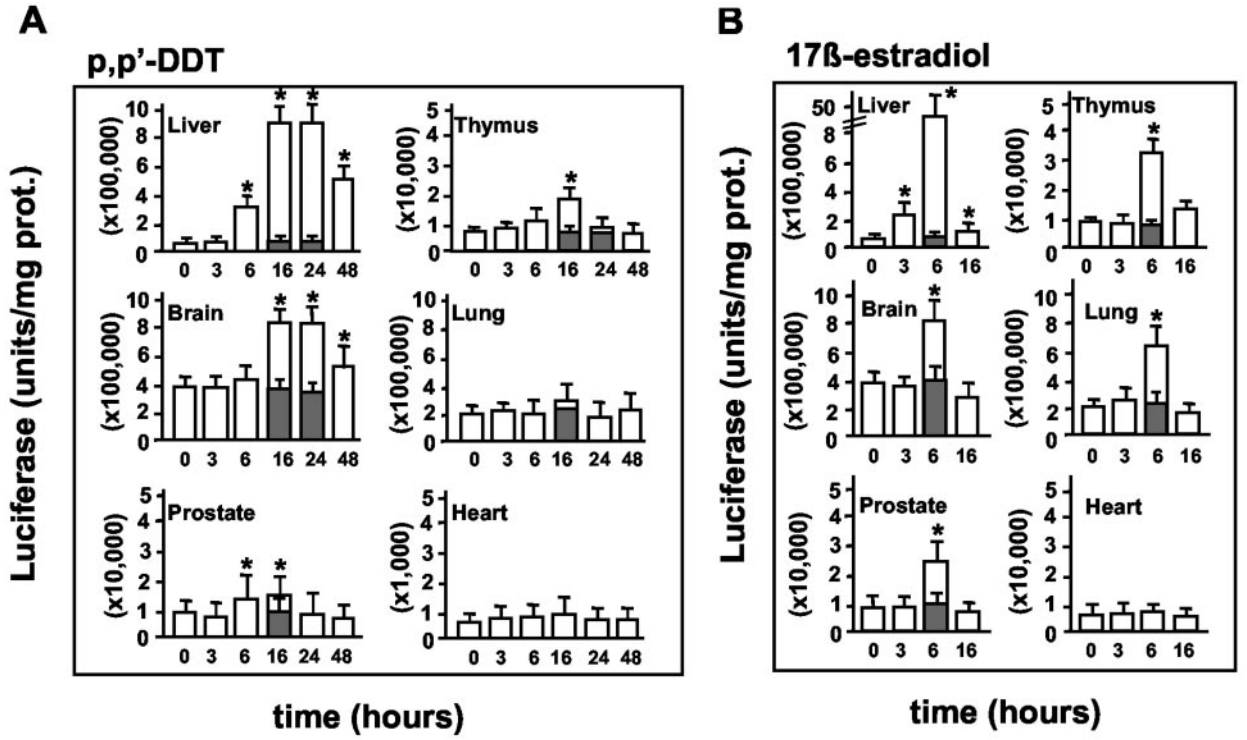

FIG. 1. 17β-E2 and p,p'-DDT effects on luciferase expression in ERE-tkLUC mice. Two-month-old mice were fed an estrogen-free diet for 2 d before treatments. Vegetal oil (*shaded bars*) or an oil solution of p,p'-DDT (5000 μg/kg) (A, *clear bars*) or 17β-E2 (50 μg/kg) (B, *clear bars*) were administered ip at time 0. Controls (vehicle-treated animals) at defined time points are indicated as *shaded bars*. Animals were killed at the indicated time-points, and tissues were dissected and stored at −80 C until assayed. Luciferase activity, measured in tissue extracts, was expressed as relative light units normalized on protein concentration. The experiments were repeated five times, with a total of nine animals per group. *Bars* represent the average ± SEM. *, $P < 0.05$, as compared with the control.

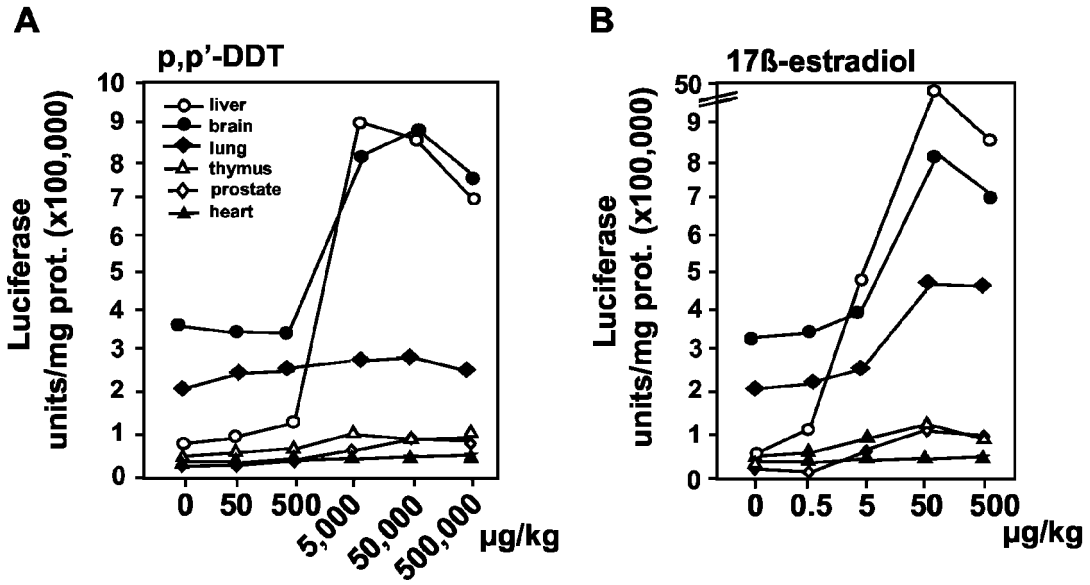

FIG. 2. Dose response analysis. ERE-tkLUC mice were treated ip with oil; with p,p'-DDT at the doses of 50, 500, 5,000, 50,000, and 500,000 μg/kg; or 17β-E2 at the doses of 0.5, 5, 50, and 500 μg/kg for 16 or 6 h, respectively. Luciferase activity was determined as specified in the previous figure. The experiments were repeated three times, with a total of six animals per group; data represent the mean of all the experiments done. Values are plotted on a logarithmic scale.

p,p'-DDT activity requires ER

Previous studies on DDT isomers and metabolites have questioned whether the less estrogenic DDT isomer p,p′-DDT was really acting through ERs (17, 29), or better via aromatase induction of its major metabolite p,p′-DDE (30) or by stimulating growth factor receptor tyrosine kinase activity

(31). Thus, to assess whether the described effects of p,p′-DDT on the ERE-luciferase transgene were mediated by the ER, 2-month-old male mice were treated with the compound together with the selective ER antagonist ICI-182,780. To ensure the blockade of ER, ICI-182,780 was injected at the dose of 20 mg/kg, 1 h before (10 mg/kg) and 6 h after (10 mg/kg) p,p′-DDT (at the dose of 5000 μg/kg). Luciferase activity was measured in liver, brain, prostate, and thymus, 16 h after injection of p,p′-DDT. As shown in Fig. 4, the induction of the reporter was efficiently blocked by ICI-182,780 in all the tissues analyzed. ICI-182,780 alone did not elicit any significant effect, suggesting its lack of toxicity on the system. These data indicate that p,p′-DDT activity in these tissues requires ER.

o,p′-DDT isomer displays an antagonist activity on the liver ERs

p,p′-DDT represents the most abundant isomer (80%) of the technical grade DDT. We then tested also the activity of the less abundant, but more estrogenic o,p′-DDT. In all the tissues tested, except the liver, o,p′-DDT induced maximal luciferase accumulation 16 h after treatment (Fig. 5A). As observed with the p,p′-DDT isomer, the maximal effect was at the dose of 5000 μg/kg (not shown). Interestingly, at the same concentrations used to activate the reporter in the brain, thymus, and prostate, o,p′-DDT inhibited luciferase accumulation in the liver. To assess whether the decreased transgene expression observed in liver was attributable to trans-repression or blockade of ER activity,

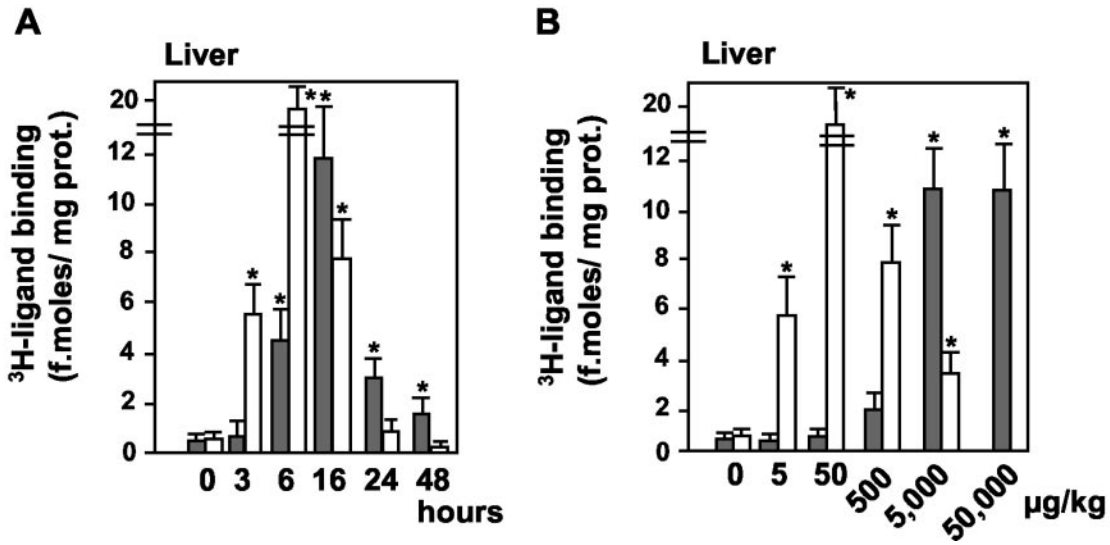

FIG. 3. Effect of p,p′-DDT administration on progesterone receptors in ERE-tkLUC mice liver. Mice were treated as specified in Figs. 1 and 2; doses of 17β-E2 (*open bars*) or p,p′-DDT (*shaded bars*) are plotted on a logarithmic scale. PR binding was measured in cytosol of mouse liver, by the charcoal-dextran method, using [^{3}H]ORG2058 as radioligand and progesterone as cold competitor. Values are expressed as femtomoles per milligram of cytosolic protein. *Bars* represent the average ± SEM of two individual experiments, each performed in triplicate. *, $P < 0.05$, as compared with the control. prot., Protein.

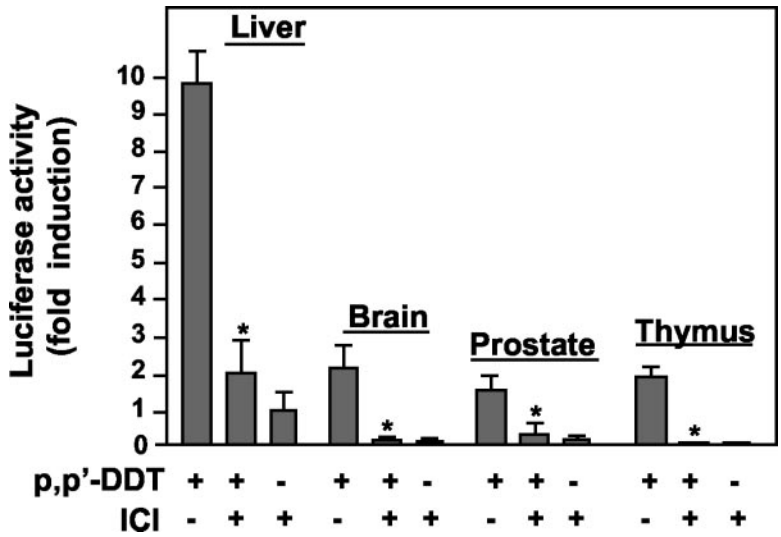

FIG. 4. p.p′-DDT induction of luciferase expression is blocked by the ER-specific antagonist ICI-182,780. A total of 10 mg/kg ICI-182,780 was injected ip, 1 h before and 6 h after a single dose of p,p′-DDT (5000 μg/kg). Mice were killed 16 h after p,p′-DDT administration. Luciferase activity was normalized for protein content; fold induction was calculated with respect to values measured in control mice (oil-injected). The graph represents the data average of three separate experiments, each performed on groups of two mice each. *, $P < 0.05$ *vs.* p,p′-DDT-treated mice.

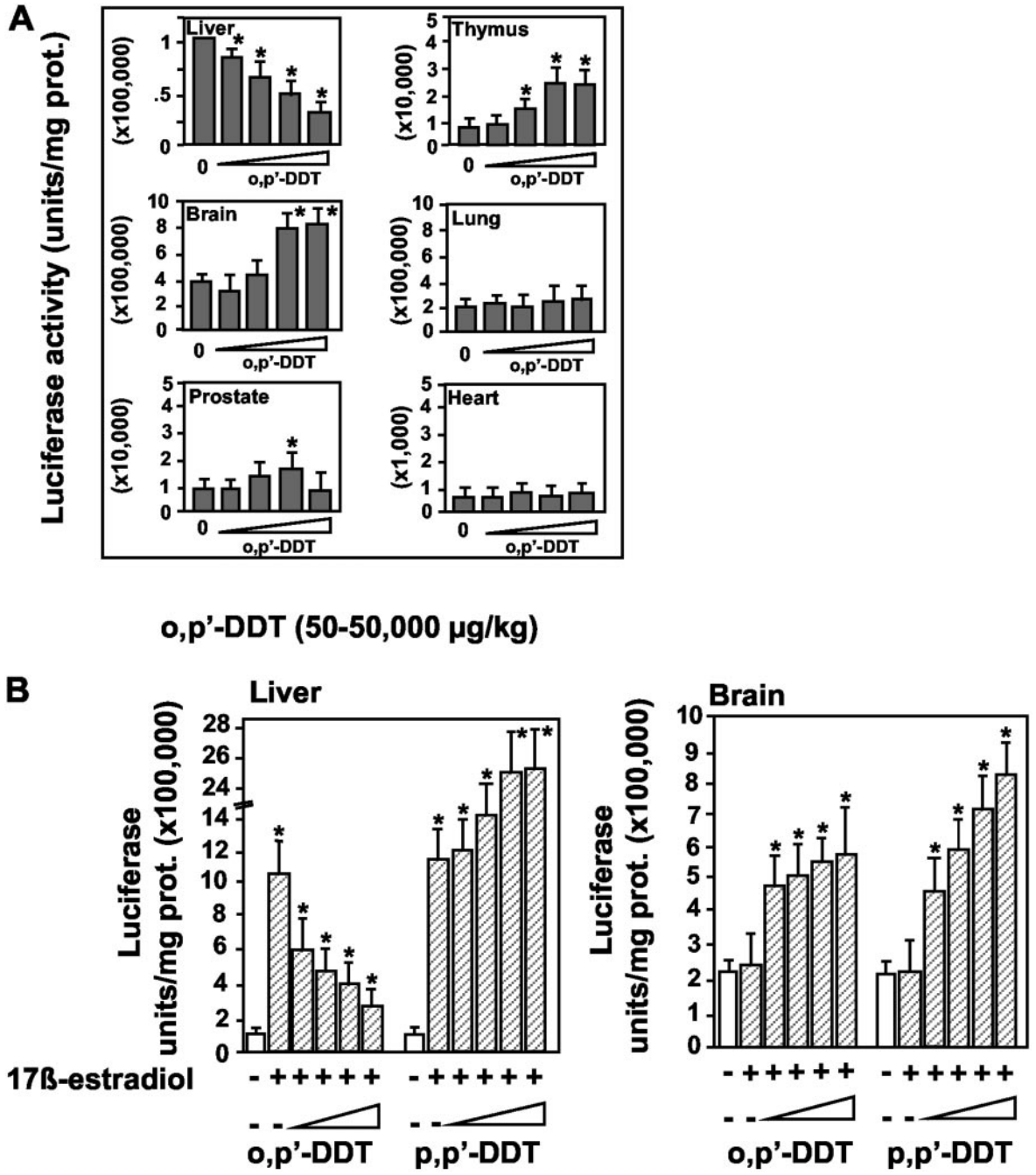

FIG. 5. o,p'-DDT activates ER transcriptional activity in all the tissues but not in liver. A, Adult mice were injected with oil or an oil solution of o,p'-DDT at increasing concentrations (50–500 to 5,000–50,000 μg/kg). Mice were killed 16 h after treatment. B, Competition experiments of the DDT isomers with 17β-E2. Two-month-old mice, fed with an estrogen-free diet for 2 d before treatments, were injected ip with 17β-E2 (50 μg/kg) alone or with four concentrations of p,p'-DDT or o,p'-DDT (50–500 to 5,000–50,000 μg/kg). Controls were injected with vegetal oil. Luciferase activity was measured in liver and brain of mice killed 16 h after treatment. Doses of p,p'-DDT and o,p'-DDT are plotted on a logarithmic scale. The experiments were repeated three times, with a total of six animals per group. *Bars* represent the average ± SEM of two individual experiments, each performed in triplicate. *, $P < 0.05$, as compared with the control.

we treated adult male mice with either a fixed amount of 17β-E2 (50 μg/kg) alone or with increasing doses of o,p′-DDT or p,p′-DDT for 16 h. Figure 5B shows that the coadministration of 17β-E2 and o,p′-DDT resulted in a significant decreased luciferase activity in liver, but not in brain, where the presence of the o,p′-DDT isomer increased the natural hormone effect.

Activity of ER in 4-d-old mice nursed by p,p'-DDT- and o,p'-DDT-treated mothers

Because exposure to DDT has been shown to be particularly critical during perinatal life (23, 32, 33) and because DDT contaminates milk (5, 25, 34, 35), we used the ERE-tkLUC model to investigate whether DDT was activating ERs

in newborns breast-fed by mothers exposed to this compound. Lactating transgenic mothers were given a single ip injection of p,p′-DDT or o,p′-DDT (50 mg/kg body weight) at d 4 after delivery. Suckling pups were killed 16 h after treatment of the mothers, and luciferase activity was measured in their livers and brains. The amount of milk the pups had sucked was hypothesized by weighing the stomachs. In pups nursed by the DDT-treated mothers, the stomach weighed 40% less than controls. Despite that, the amount of DDT in the milk was sufficient to modulate ERs, as shown by luciferase enzymatic activity (Fig. 6). Treatment of the lactating mothers with both DDT isomers resulted in an increase of luciferase activity in the pups' brains. In liver, the effect of the two isomers reflected what was already described in adult mice: p,p′-DDT caused an increase, and o,p′-DDT caused a decrease, of luciferase activity, although with a different magnitude than with the adults. To assess whether these differences in reporter induction could be ascribed to lower levels of circulating DDT in pups, p,p′-DDT was measured in serum of lactating mothers and suckling mice and in milk, by GC-MS, at the time point showing maximal induction. In animals treated with vehicle, p,p′-DDT was undetectable. In the serum of lactating mice, we measured an average of 1,460 ± 266 ng/ml, 16 h after injection. Interestingly, at the same time-point, the concentration of p,p′-DDT in milk was significantly higher than in serum (53,790 ± 3,550 ng/g milk fat). In the serum of pups, p,p′-DDT content was of 208 ± 19 ng/ml (Table 1). As shown in Fig. 6, this p,p′-DDT concentration was sufficient to activate the reporter, both in liver and brain. These results show

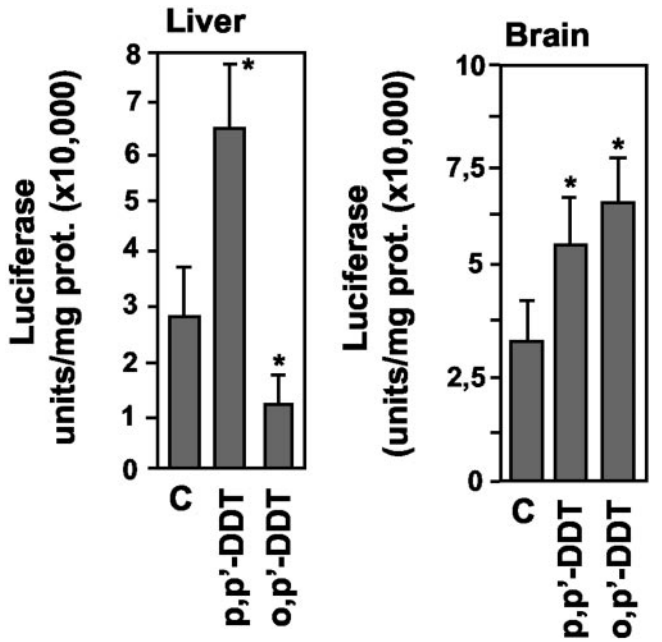

FIG. 6. Effects of DDT isomers in the suckling mice. Lactating mothers, maintained on a normal diet, were injected with 50 mg/kg body weight of p,p′-DDT, o,p′-DDT or vehicle as control. Mothers were treated at d 4 after delivery, and pups were killed 16 h later. Luciferase activity was normalized to protein content and monitored in liver and brain of the newborn. Each *column* is the mean ± SEM of five mice/group from two separate experiments. *, $P < 0,05$, compared with the appropriate control.

TABLE 1. Levels of p,p′-DDT in milk and serum of adult and suckling mice

Treatment	Lactating mother		Suckling mice
	Serum (ng/ml)	Milk (ng/g fat)	Serum (ng/ml)
Control	n.d.	n.d.	n.d.
p,p′-DDT	1,460 ± 266	53,790 ± 3,550	208 ± 19

n.d., Not detected. Data represent the mean ± SEM of two individual experiments, each on three animals.

that, in suckling newborns, reporter activity is regulated by concentrations of p,p′-DDT similar to those often reported in serum of humans in exposed populations (36).

Discussion

By the use of a novel reporter mouse, we here provide evidence of the activity of p,p′-DDT and o,p′-DDT in mouse nonreproductive tissues and prostate. Our data demonstrate a differential isomer-specific activity in liver but not in all the other organs tested. We also show that this isomer-specific effect can be observed in newborn mice lactating from a mother exposed to DDT.

The present study demonstrates, for the first time, that DDT isomers modulate ER activity in nonreproductive organs, such as liver, brain, and thymus. So far, little is known with regard to the effects of these compounds in nonreproductive organs. Previous epidemiological studies have shown effects of DDT on liver and the central nervous system (CNS) (8, 10). Toxicity in liver has also been experimentally reproduced by Takayama *et al.* (11) in nonhuman primates. The experimental data on the effects on the CNS *in vivo* are few (33, 37) and relate to the altered behavior of the exposed animals. Our data provide the basis for the understanding of the mechanism of toxicity of these compounds in liver and CNS. Furthermore, by showing an effect in prostate, and particularly in thymus, our findings suggest novel sites for potential toxic effects of DDT. This observation is worth pursuing in more detailed experimental or epidemiological studies.

In this initial systematic analysis of the effects of DDT isomers on ERs *in vivo*, two observations need to be underlined. First, both isomers were inactive in lung, a tissue known to express ERβ and clearly responsive to 17β-E2. Second, in liver, the two isomers display opposite effects, p,p′-DDT being an ER agonist, and o,p′-DDT an ER antagonist. Liver is known to express predominantly ERα, whereas lung expresses ERβ. These observations lead us to hypothesize that DDT is inactive in tissues expressing only ERβ and may have a differential effect when only ERα is expressed. Supporting this hypothesis is the fact that also in prostate, an organ with predominant expression of ERβ, the luciferase induction by DDT is weaker than in the other responsive tissues. This might have important implications for the evaluation of the toxicity profile of different organochlorine pesticides. Remaining unclear is the significance of the delayed response of ERs to DDT; the maximal luciferase and PR response was in fact observed at 6 and 16 h after injection of 17β-E2 and DDT, respectively. This could be attributable to a slower kinetic of distribution of DDT isomers from the site of injection.

The data here presented demonstrate that the reporter mouse we used is a very promising system for acquiring a global perspective of ED activity in a mammalian organism. As described in the previous publication (24), and here with the analysis of estrogen and DDT effects on an endogenous target for estrogens (PR), the measure of luciferase seems to fully reflect the activity of ERs *in vivo*. The present ERE-tkLUC mouse can only allow monitoring of the sum of ERα and ERβ transcriptional activities. Novel models obtained by breeding the ERE-tkLUC mice into ERα and ERβ knockout mice, will enable the study of the effects on each ER receptor subtype.

Recently, Nagel *et al.* (38) also proposed the use of reporter mice in the study of the effects of xenobiotics. This model, however, has several advantages, with respect to Nagel's. In fact, the presence of insulator sequences surrounding the reporter element prevents position effects and ensures the expression and full responsiveness of the reporter in all the mouse cells. Furthermore, the choice of luciferase as reporter grants a dynamic analysis of the state of ER activity. This is because the wild-type luciferase has proven to have a fast turnover when expressed in mammalian tissues (half-life of 2–3 h). Finally, luciferase activity can be monitored *in vivo* by photon imaging. This enables one to obtain a global, whole-body view of the state of ER activity in time, within the same animal.

When using a reporter mouse, the major concern regards the limited sensitivity of the animal system. Indeed, in the ERE-tkLUC mouse, the induction of luciferase was triggered at DDT isomer concentrations almost one order of magnitude lower than those necessary to induce the uterotropic effect in prepuberal female mice (18, 39). We also show that o,p'-DDT, at a dose known to interfere with animal fertility (50 μg/kg), significantly affects ER response. In female mice with a single injection of 50,000 μg/kg DDT, we reached serum concentrations similar to those reported in exposed populations (25, 36, 40); and at that dosage, the effect of the toxic compound on luciferase is very strong.

Most interesting are the data on suckling mice. In these mice, breast fed by DDT-exposed mothers, we measured a blood concentration of the toxic agent (208 ng/ml) similar to values of DDT and other organochlorine compounds [polychlorinated biphenyls (PCBs)] found in blood of peoples living in industrialized areas (25, 36). This would indicate that the model is adequate for studies on the activity of xenobiotics concentrations comparable with those found in environmentally exposed individuals. The possibility of following the effects of DDT during development and lactation in all the mouse tissues will finally provide functional data on the effects of pesticides on reproductive and nonreproductive organs, still incomplete for newborn and juvenile mammals. Therefore, different from the uterotropic assay, which is the most used *in vivo* model so far used for the study of toxic compounds active on ERs, the model system we generated has the necessary sensitivity and target specificity to provide information on the tissue-selective agonist/antagonist effect of EDs.

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References

1. **Vos JG, Dybing E, Greim HA, Ladefoget O, Lambre C, Tarazona JV, Brandt I, Vethaak AD** 2000 Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Crit Rev Toxicol 30:71–133
2. **Sparling DW, Fellers GM, McConnell L** 2001 Pesticides and amphibian population declines in California, USA. Environ Toxicol Chem 20:1591–1595
3. **Borrel A, Cantos G, Pastor T, Aguilar A** 2001 Organochlorine compounds in common dolphins (Delphinus delphis) from the Atlantic and Mediterranean waters of Spain. Environ Pollut 114:265–274
4. **Desaulniers D, Leingartner K, Russo J, Perkins G, Chittim BG, Archer MC, Wade M, Yang J** 2001 Modulatory effects of neonatal exposure to TCDD, or a mixture of PCBs, p, p'-DDT, and p, p'-DDE, on methylnitrosourea-induced mammary tumor development in the rat. Environ Health Perspect 109:739–747
5. **Longnecker MP, Klebanoff MA, Zhou H, Brock JW** 2001 Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. Lancet 358:110–114
6. **Korrick SA, Chen C, Damokosh AI, Ni J, Liu X, Cho SI, Altskul L, Ryan L, Xu X** 2001 Association of DDT with spontaneous abortion: a case control study. Ann Epidemiol 11:481–486
7. **Simonich SL, Hites RA** 1995 Global distribution of persistent organochlorine compounds. Science 269:1851–1854
8. **van Wendel de Joode B, Wesseling C, Kromhout H, Monge P, Garcia M, Mergler D** 2001 Chronic nervous-system effects of long-term occupational exposure to DDT. Lancet 357:101–1016
9. **Ruelhman DO, Steinert JR, Valverde MA, Jacob R, Mann GE** 1998 Environmental estrogenic pollutants induce acute vascular relaxation by inhibiting L-type Ca2+ channels in smooth muscle cells. FASEB J 12:613–619
10. **Cocco P, Kazerouni N, Zahm SH** 2000 Cancer mortality and environmental exposure to DDE in the United States. Environ Health Perspect 108:1–4
11. **Takayama S, Sieber SM, Dalgard DW, Thorgeirsson UP, Adamson RH** 1999 Effects of long-term oral administration of DDT on non-human primates. J Cancer Res Clin Oncol 125:219–225
12. **Vine MF, Stein L, Weigle K, Schroeder J, Degnan D, Tse CK, Hanchette C, Backer L** 2000 Effects on the immune system associated with living near a pesticide dump site. Environ Health Perspect 108:1113–1124
13. **Jarukamjorn K, Sakuma T, Yamamoto M, Ohara A, Nemoto N** 2001 Sex-associated expression of mouse hepatic and renal CYP2B enzymes by glucocorticoid hormones. Biochem Pharmacol 62:161–169
14. **Cheek AO, Kow K, Chen J, McLachlan JA** 1999 Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. Environ Health Perspect 107:273–278
15. **Leffers H, Naesby M, Vendelbo B, Skakkebaek NE, Jorgensen M** 2001 Oestrogenic potencies of zeranol, oestradiol, diethylstilboestrol, bisphenol A and genistein: implications for exposure assessment of potential endocrine disrupters. Hum Reprod 16:1037–1045
16. **Strunck E, Stemmann N, Hopert A-C, Wunsche W, Frank K, Vollmer G** 2000 Relative binding affinity does not predict biological response to xenoestrogens in rat endometrial adenocarcinoma cells. J Steroid Biochem Mol Biol 74:73–81
17. **Kuiper G, Lemmen J, Carlsson B, Corton JC, Safe S, van der Saag P, van der Burg B, Gustafsson JA** 1998 Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139:4252–4263
18. **Newbold RR, Jefferson WN, Padilla-Banks E, Walker WR, Pena DS** 2001 Cell response end-points enhance sensitivity to the immature mouse uterotrophic assay. Reprod Toxicol 15:242–252
19. **Thompson RS, Hess DL, Binkerd PE, Hendrickx AG** 1981 The effects of prenatal diethylstilbestrol exposure on the genitalia of pubertal *Macaca Mulatta* II male offspring. J Reprod Med 26:309–316
20. **Stillman RJ** 1982 *In utero* exposure to diethylstilbestrol: adverse effects on the reproductive tract and reproductive performance in male and female offspring. Am J Obstet Gynecol 142:905–921
21. **Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette Jr LJ, Jegou B, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer O, Muller J, Rajpert-De Meyts E, Scheike T, Sharpe RM, Sumpter JS, Skakkebaeck NE** 1996 Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104(Suppl 4):741–803
22. **Palanza P, Parmigiani S, Liu H, wom Saal FS** 1999 Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and o, p'-DDT alters aggressive behavior of male and female house mice. Pharmacol Biochem Behav 64:665–672
23. **Jonsson CJ, Lund BO, Bergman A, Brandt I** 1992 Adrenocortical toxicity of

3-methylsulphonyl-DDE; 3: studies in fetal and suckling mice. Reprod Toxicol 6:233–240
24. **Ciana P, Di Luccio G, Belcredito S, Pollio G, Vegeto E, Tatangelo L, Maggi A** 2001 Engineering of a mouse for the "in vivo" profiling of estrogen receptor activity. Mol Endocrinol 15:1104–1113
25. **Smith D** 1999 Worldwide trends in DDT levels in human breast milk. Int J Epidemiol 28:179–188
26. **Bradford MM** 1976 A rapid and sensitive methods for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem 72:248–251
27. **de Vet JR, Wood KV, De Luca M, Helinski DR, Subramani S** 1987 Firefly luciferase gene: structure and expression in mammalian cells. Mol Cell Biol 7:725–737
28. **Scatchard G** 1947 The attraction of proteins for small molecules and ions. Ann NY Acad Sci 51:660–668
29. **Chen CW, Hurd C, Vorojeikina DP, Arnold SF, Notides AC** 1997 Transcriptional activation of the human estrogen receptor by DDT isomers and metabolites in yeast and MCF-7 cells. Biochem Pharmacol 53:1161–1172
30. **You L, Sar M, Bartolucci E, Ploch S, Whitt M** 2001 Induction of hepatic aromatase by p, p′-DDE in adult male rats. Moll Cell Endocrinol 178:207–214
31. **Shen K, Novak RF** 1997 DDT stimulates c-erbB2, c-met, and STATS tyrosine phosphorylation, Grb2-Sos association, MAPK phosphorylation and proliferation of human breast epithelial cells. Biochem Biophys Res Commun 231:17–21
32. **Kihlstrom JE, Lundberg C, Orberg J, Danielsen PO, Sydhoff J** 1975 Sexual functions of mice neonatally exposed to DDT or PCB. Environ Physiol Biochem 5:54–57
33. **Palanza P, Morellini F, Parmigiani S, wom Saal FS** 1999 Prenatal exposure to endocrine disrupting chemicals: effects on behavioral development. Neurosci Biobehav Rev 23:1011–1027
34. **Torres-Arreola L, Lopez-Carrillo L, Torres-Sanchez L, Cebrian M, Rueda C, Reyes R, Lopez-Cervantes M** 1999 Levels of dichloro-dyphenyl-trichloroethane (DDT) metabolites in maternal milk and their determinant factors. Arch Environ Health 54:124–129
35. **Pohl HR, Tylenda CA** 2000 Breast-feeding exposure of infants to selected pesticides: a public health viewpoint. Toxicol Ind Health 16:65–77
36. **Rothman N, Cantor KP, Blair A, Bush D, Brock JW, Helzisouer K, Zahm SH, Needham LL, Pearson GR, Hoover RN, Comstock GW, Strickland PT** 1997 A nested case-control study of non-Hodgkin lymphoma and serum organochlorine residues. Lancet 350:240–244
37. **Palanza P, Parmigiani S, vom Saal FS** 2001 Effects of prenatal exposure to low doses of diethylstilbestrol, o, p′-DDT and nethoxychlor on postnatal growth and neurobehavioral development in male and female mice. Horm Behav 40:252–265
38. **Nagel CS, Hagelbarger JL, McDonnell DP** 2001 Development of an ER action indicator mouse for the study of estrogens, selective ER modulators (SERMs), and xenobiotics. Endocrinology 142:4721–4727
39. **Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL** 1996 Assessing environmental chemicals for estrogenicity using a combination of "in vitro" and "in vivo" assays. Environ Health Perspect 104:1296–1300
40. **Walisevski SM, Aguirre AA, Silva CS, Siliceo J** 2001 Organoclorine pesticide levels in maternal adipose tissue, blood, serum, umbilical blood and milk from inhabitants of Veracruz, Mexico. Arch Environ Contam Toxicol 40:432–438